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GB 1471976
GB 1305923
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(54) A method of aligning particles in a moving fluid sample

(57) A method is provided for aligning particles in a fluid sample 26 which are moving in a direction through a flow cell 10. A sheath fluid 22 also flows through the flow cell 10 parallel to the direction of flow of the sample fluid 26. The flow rate of the sheath fluid 22 or the sample fluid 26 can be adjusted such that the particles are aligned substantially with their minimum cross-sectional area extended transverse to the direction of flow and with their maximum cross-sectional area extended substantially parallel to the width of flow cell 10 and with the centers of mass A, B, C of the particles of fluid sample lying substantially in one plane 120. With the centers of mass A, B, C of the particles of the fluid sample substantially in one plane 120, a microscope 30 can be focused on the one plane to view the particles. In addition, the microscope 30 can be focused on a plane 122 different from the one plane 120 in order to achieve optical separation of the particles.

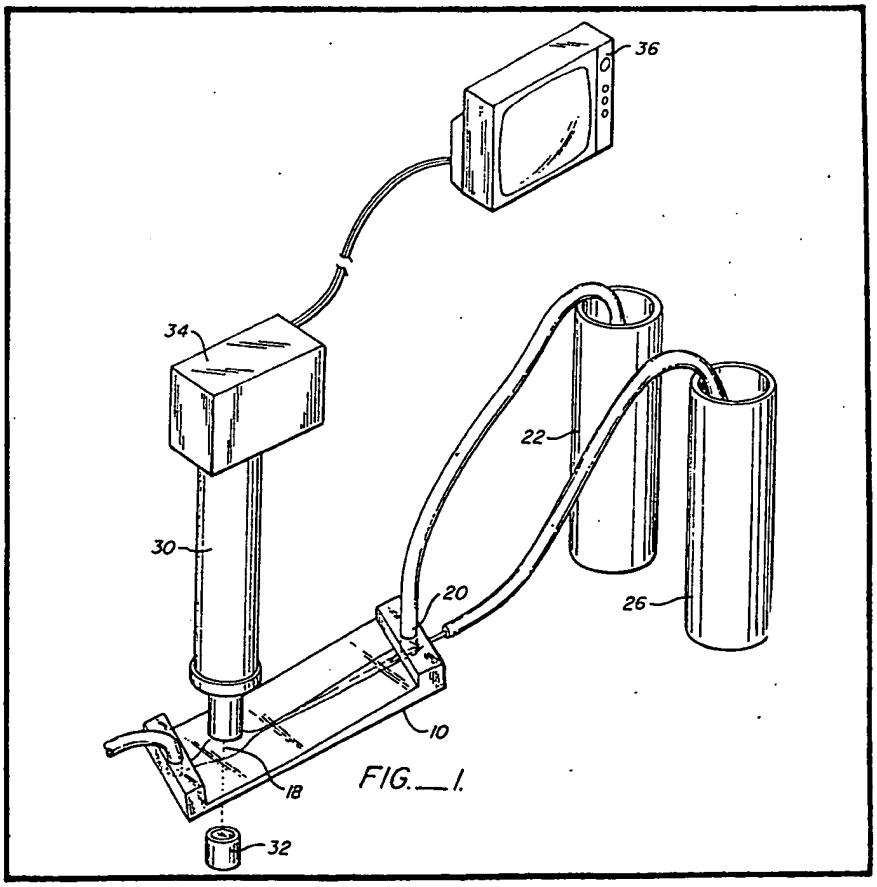

FIG. 1.

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The drawing(s) originally filed was/were informal and the print here reproduced is taken from a later filed formal copy.

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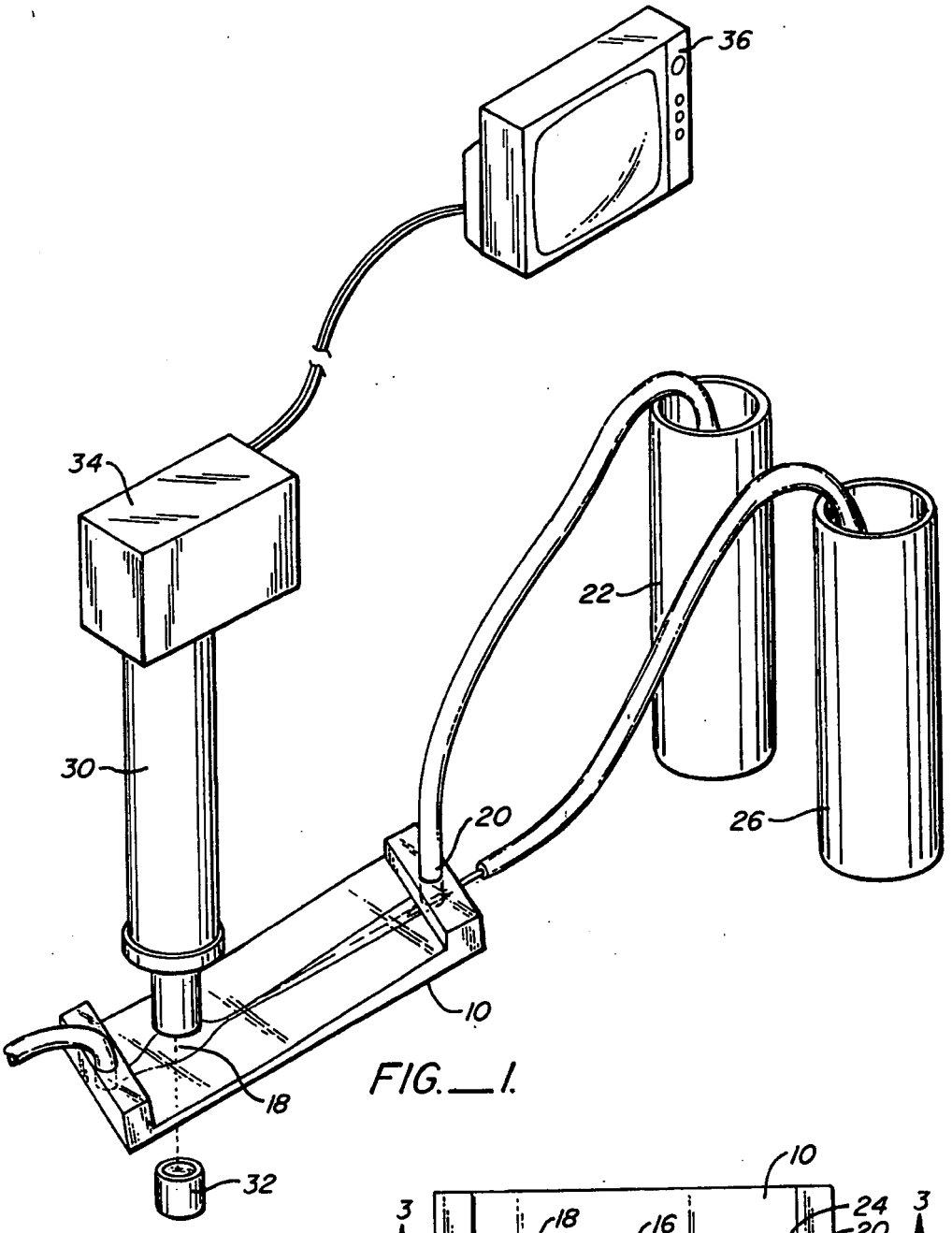

FIG. 1.

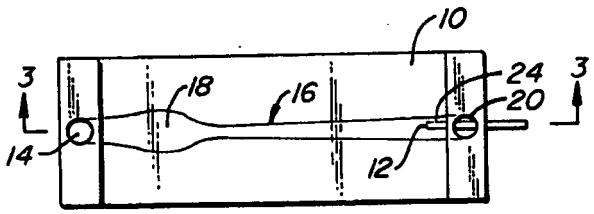

FIG. 2.

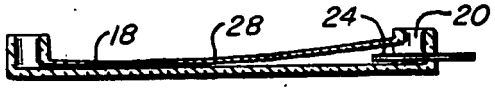

FIG. 3.

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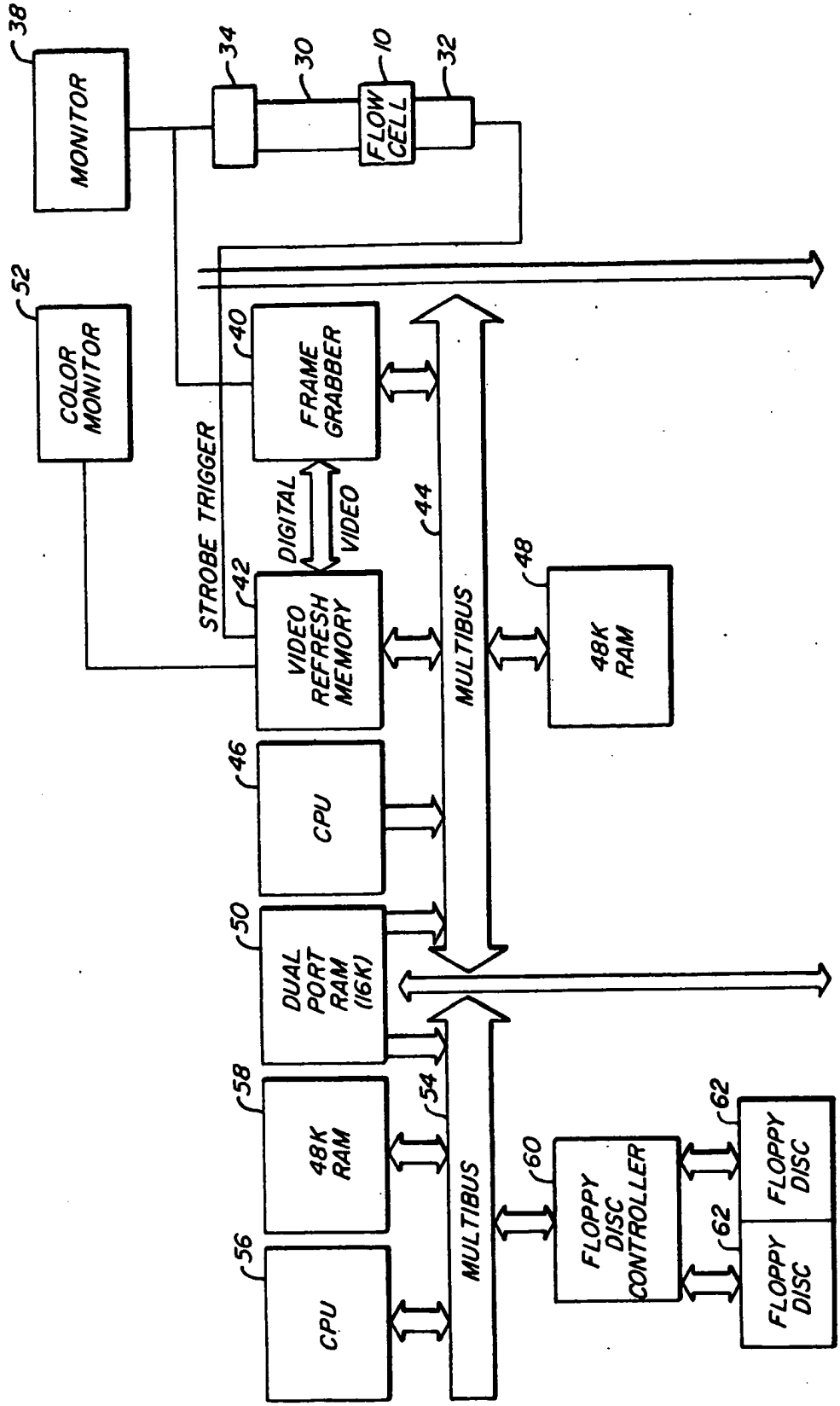

FIG. 4.

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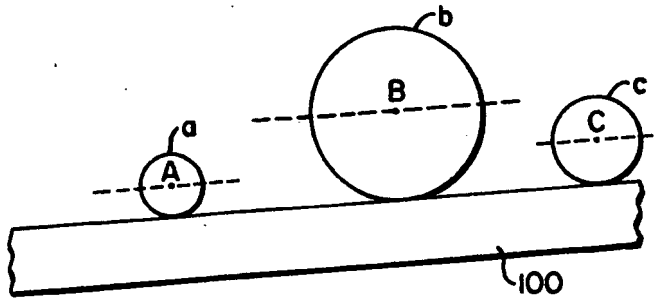

(PRIOR ART)

FIG. 5.

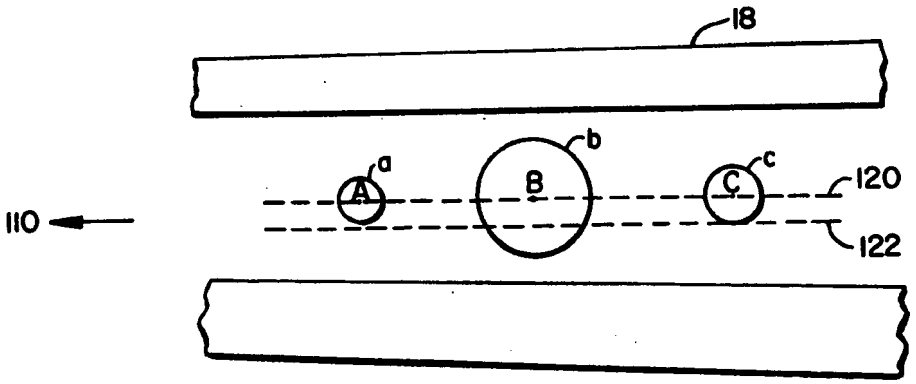

FIG. 6.

SPECIFICATION

A method of aligning particles in a moving fluid sample

Substantial advances have been made in automating the job of counting blood cells in a serum sample. The most well-known instrument for performing blood counts is the so-called Coulter counter in which blood cells are passed in single file through an orifice and detected and counted by the manner in which they change the electric properties at the orifice. Up until the present time, however, there has been no automated equipment available for analyzing and evaluating the multiple cells, such as normal cells, target cells, sickle cells, etc which may be founded in a flowing stream of a given blood sample. Thus, where multiple cell information of this type if desired, the standard commercial way of obtaining it is by preparing a microscope slide with the cells fixed on an image plane and having a human operator or pattern recognition machine count statistically significant numbers of the cells as the cells are observed one-at-a-time on the slide through a microscope. See US Patent No. 4,175,860 and 4,199,748.

Some attempts have been made in recent years to provide optical analysis of particles flowing in a flow stream. For instance, Kay, et al, Journal of *Histochemistry and Cytochemistry*, Volume 27, page 329 (1979) shows a Coulter type orifice for moving cells in single file with the cells magnified on a vidicon. Additionally, Kachel, et al., *Journal of Histochemistry and Cytochemistry*, Volume 27, page 335, shows a device for moving cells in single file through a microscopic area where they are photographed. While these workers have done some work in automating particle analysis in single file, no successful work has been reported where automated particle analysis was accomplished in a flowing stream without the requirement of arranging the particles into a single file stream. See, for instance, *Flow Cytometry and Sorting*, Melaned, et al., John Wiley and Sons 1979, Chapter 1; see also U.S. Patent No. 3,819,270.

Summary of the invention

In accordance with the present invention, a method is provided for aligning particles in a fluid sample which is moving in a direction through a flow cell. The cell has a width and a thickness, each of which is perpendicular to the direction of flow. Each of the particles of the fluid sample has a center of mass. The method of the present invention comprises the steps of conveying the fluid sample through the flow cell. A sheath fluid flows through the flow cell in the direction of flow, and is to one side of the fluid sample. The flow rate of the sheath fluid is adjusted, such that the particles are aligned substantially with their minimum cross-sectional area extended transverse to the direction of flow and with their maximum cross-sectional area extended substantially parallel to the width and with the centers of mass of the particles all lying substantially in one plane.

With the centers of mass of the particles substantially in one plane, the particles can be viewed by a microscope means focused on the one plane. Furthermore, the particles can be optically separated by focusing the microscope means on a plane other than the one plane, such that particles of one size are defocused and particles of another size are substantially in focus.

Brief description of the drawings

Fig. 1 is a perspective view of an apparatus for examining a flow stream in accordance with this invention.

Fig. 2 is a plan view of the flow chamber in Fig. 1.

Fig. 3 is a cross-sectional view of the apparatus of Fig. 2 taken on the plane indicated at 3—3.

Fig. 4 is a schematic diagram of the electronic processor employed by the apparatus of Fig. 1.

Fig. 5 is a greatly exaggerated side view of the prior art method of mounting particles on a microscope for examination.

Fig. 6 is a greatly exaggerated side view of the flow of particles in the apparatus described herein in accordance with the method of the present invention.

Detailed description of the drawings

Referring now in detail to the drawings, and particularly to Fig. 1, the apparatus shown therein includes a body 10 containing a flow chamber having an inlet 12 for a fluid sample, such as blood or urine, and an outlet 14 with a passageway 16 extending between them past an imaging area 18. The passagway 16 has an inlet with a conduit 20 adapted to be connected to a volume of saline solution 22. As illustrated in Figs. 2 and 3, the inlet 12 for the fluid sample has a needle 24 in the passageway 16 downstream from the conduit 20 with the needle 24 connected to a container 26 adapted to hold the fluid sample to be analyzed.

From the inlet 12 to the imaging area 18, the cross-sectional area becomes progressively smaller. The thickness also decreases from the inlet 12 to the imaging area 18. The width decreases from the inlet 12 and then increases substantially to the imaging area 18. Thus, as illustrated in Figs. 2 and 3, the passageway 16 has a width and depth of about 5,000 microns at the inlet 12 and a width and depth of about 500 microns at midpoint 28, and a depth of 100 microns with a width exceeding 5,000 microns at the examination area 18.

It will be appreciated that the flow stream through the examination area 18 can be many times deeper than the largest cells with the width many times wider than the widest particles, such as more than one hundred times as wide as the widest particle. However, with the flow passageway shaped in this way, the stream entering through the opening 12 is confined to a stable flow path of minimum shear in the

examination area 18. Preferably, the cross-sectional area of minimum shear is not substantially larger than the minimum cross-sectional area of the particles whereby the particles are aligned in the flow stream with their minimum cross-sectional area extended transverse to their direction of flow and their maximum cross-sectional area parallel to the width (i.e. visible in the plane of Figure 2). The term "minimum shear" is used herein to mean "minimum velocity gradient" so that a particle moving in the stream tends to align itself with the direction of the stream much as a log floating down a river will align itself with the direction of flow where there is a flow gradient. The flow characteristics in the passageway 16 may be controlled by adjusting the fluid pressure in containers 22 and 26 either automatically or by adjusting the static heights thereof.

A microscope 30 is focused on the examination area 18 and the examination area 18 is illuminated from below by a strobe light 32 which is preferably a U.S. Scientific Instrument Corporation Model 3018 containing a 2UP1.5 lamp. The output of the microscope 30 is focused on a CCD camera 34 which is preferably a CCD camera model number TC1160BD manufactured by RCA Corporation. The output of the CCD camera 34 is converted to a series of still frame images, and suitable electronic processors are employed for evaluating those images. One processor which may be employed is the processor marketed as Image Analysis System Model C-1285 by Hamamatsu Systems, Inc., Waltham, Massachusetts. Preferably, the output of the CCD camera 34 is connected to an electronic processor 36 which is illustrated in greater detail in Fig. 4 and includes a black and white television monitor 38 and a frame grabber 40 which stores still frame images of the subject viewed by the CCD camera. The frame grabber 40 is preferably a Model FG08 frame grabber made by the Matrox Corporation of Montreal, the output of which is supplied to a video refresh memory 42 Model RGB 256 made by Matrox Corporation which are both coupled to the multibus 44 of the central processing unit 46 which is preferably an Intel 80/20 computer. The multibus 44 is also coupled to a 48K random access memory 48 of Electronic Solutions, Inc., and a 16K dual port random access memory 50 Model RM 117 of Data Cube Corporation. The output of the video refresh memory is also coupled to a color monitor 52 which may be used to provide digitally enhanced video images of individual still frames for human examination.

The second output of the dual port ram 50 is connected to a multibus 54 which is connected to an Applied Micro Devices central processing unit 56, a 48K random access memory of Electronic Solutions, Inc. 58 and removable storage in the form of a floppy disc controller 60, such as an Advanced Micro Devices Model 8/8 and two units of Shugart floppy disc storage 62.

A wide variety of programming may be employed for processing pictures with the apparatus of Fig. 4 depending upon the particular task which user wishes to perform.

As mentioned above, the programming of the Hamamatsu System 1285 may be employed. Preferably, however, the programming is performed as follows:

The tasks are first divided into those which must address each pixel in a given image and those which only address a small subset of the total. Since much time will be spent in the first class of tasks, they are programmed in assembly language on the interface processor 46 (the Intel 80/20 in Fig. 4). The output of these operations are then transferred to the host machine 56 via the dual ported ram 50. On the host side, almost all of the necessary programming is more suitably done in a high level language such as Pascal (Basic or Fortran could in principal be used also). The types of tasks that are done in the assembly language includes greyscale transformations, convolutions, and greyscale histogram calculations. The types of tasks done on the hoste side include overall control of the other devices, identification and segmentation of object of interest in the field of view, calculation of parameters associated with objects thus found, and formating the output of results. Another way of considering this separation of tasks in this fashion is that tasks which must be performed at speeds faster than a human operator are done in assembly. Tasks which are either complicated or which can operate at less than the maximum speed can be programmed in the higher language. Objects are found in a field of view primarily by setting a greyscale window function for values known to be characteristic of the desired object. These values can be established by prior knowledge or by well-known histogram techniques. When a pixel belonging to an object has been located in the field of view, an edge tracing program is invoked to outline the whole object associated with that pixel. Once the edge has been found, then many relevant parameters such as location, area, integrated optical density, and various moments can easily be calculated. Probability of membership in previously defined subgroups can be determined from these derived parameters by means of standard decision theory. Definitions of cell morphology classifications are established by trained observers. These definitions are then used as the basis of the selected algorithms. Accuracy of the method is determined by comparison of machine results with those of trained observers examining the same samples. Output of the results can be programmed to be any of a variety of formats. Histograms, line plots, and tabular summaries are available for particular needs.

It is thus seen that with the flow cell 10, the stream of particles in the imaging area 18 is of two dimensions. Therefore, more than one particle can be examined in a single field, and different particles can be optically

distinguished with a number of important advantages. For instance, two cells flowing together can be optically recognized whereas a Coulter counter could recognize them as a single double-sized cell. In addition, the still frame images can be enhanced with the digital image enhancement techniques which have been developed for satellite pictures and the individual frames may be analyzed to provide data on individual cells. For a blood sample, information such as size, cross-sectional area, shape, (circular cell, target cell, sickle cell, etc), optical density, hemoglobin content on the cell basis, etc can be obtained. Not only can individual cells be analyzed and optically sorted in this way, but, additionally, when the cells are so analyzed and sorted, different types of cells may be individually counted to give automatically and at a single pass, the number of normal red cells per volume of sample, the number of target red cells per cc of sample, the number of sickled red cells per cc of sample, the number of white cells, the number of platelets, etc. per cc of sample.

Once a series of still frame images is prepared in digital form, a wide variety of very sophisticated information can be obtained about the particles in the series of images depending upon the complexity of computer equipment and software which may be used for analysis of the images.

Preferably, information derived from still frame images is combined to provide composite information reflecting the content of the multiple still frame images and/or predetermined reference images, and the composite information thus obtained may be used in a variety of ways. Thus, in simple systems, the information may be printed out, for instance, to advise a hematologist about composite measurements made from a blood sample. In more complex systems, the composite measurements may be used by process control, such as pressure in a homogenizer, temperature in a crystallizer, or nutrient feed rate in a microbial culture where the system monitors particle size or number.

Thus, it will be noted that the apparatus may be used for analysis of a variety of optically perceptible particles moving in a stream, both biological particles, such as cells in blood or cells, baceteria, casts and crystals in urine or particles in gas analyzers, etc, and the output of these measurements may be employed for process control, such as dispensing nutrients into a stream containing microorganisms as mentioned above, the control of the growth of polymers and crystals, etc.

In Figure 5, there is shown the prior art technique of examining particles of a fluid sample. Typically, the particles, such as those designated as a, b, and c, are mounted on a microscopic slide 100. Each of the particles a, b, and c has a center of mass designated as A, B, and C, respectively. It can be seen from Figure 5 that with the particles a, b, and c, all mounted on a slide 100, the centers of mass A, B, and C, do not lie in one plane. Therefore to view particles a, b, and c, the microscope must first be focused on particle a at a plane, parallel to the slide 100, passing through its center of mass A. To view particle b, the microscope must be adjusted so that it is focused on plane parallel to the slide 100 and passing through the center of mass B. Similarly, the microscope must be adjusted to view the particle c.

In contrast, in the method of the present invention, the particles are aligned as they move through the flow cell 10. Particles, a, b, and c in the fluid sample are moved in a direction designated by the arrow 110 through the flow cell 10. A sheath fluid is passed through the flow cell 10 also in the direction 110. The sheath fluid envelopes the sample fluid. The flow rate of the sheath fluid or the sample fluid or both fluids can be controlled such that the flow of the two fluids is within the laminar range, i.e. with little or no turbulence. Furthermore, the flow rate of each fluid or both is controlled causing the particles a, b, and c to be aligned substantially with the minimum cross-sectional area extended transverse to the direction 110 and with their maximum cross-sectional area extended substantially parallel to the width of flow cell 10, with the centers of mass A, B and C of the particles a, b, and c, respectively, all lying substantially in one plane 120 in the examination area 18. With the particles a, b, and c, so aligned in the examination area 18, the microscope 30 can be adjusted so that it is focusing on the one plane 120. All the particles a, b, and c, would then be in focus as viewed through the microscope 30. Alternatively, the flow rate of the sheath fluid can be changed moving the particles so as to shift the focal plane causing the particles to be in focus.

Furthermore, with the centers of mass A, B and C of the particles a, b, and c, respectively, lying substantially in one plane 120, a method of optically separating the particles can also be achieved. As shown in Figure 6, the microscope 30 can be adjusted to focus on a plane 122 which is different from the one plane 120. The plane 122 does not pass through particle a at all. As viewed through the microscope 30, particle a is out of focus. However, because particles b and c are substantially larger than particle a, the plane 122 at which the microscope 30 is focused would cause the particles b and c to be substantially in focus. Since particle a is not in focus, its greyscale level may not be sufficient to trigger the edge tracing routine of the electronic processor 36. Therefore, a shift in the focal plane to cause smaller particles to be out of focus results in an optical separation of the particles. Furthermore, it is believed that smaller particles such as bacteria are less affected by the hydrodynamics of the flow cell 10. That is, they are less influenced by the shape of the flow cell 10 to align their cross-sectional area with the flow direction. Particles, such as bacterial, are believed to be affected by Brownian movement. With the particles of the fluid sample which are affected by the hydrodynamics of the flow cell 10, lying

substantially in one plane 120, the plane at which the microscope 30 is in focus can be shifted in order to defocus the particles in the one plane 120, and focused on the bacteria in a different plane. In this matter, optical separation of the particles is also achieved.

There are a number of applications for the method of the present invention, among which is in the analysis of blood or urine as the fluid samples. With blood as the fluid sample red blood cells are smaller than white blood cells. Thus, by the method described heretofore, the red blood cell can be optically separated from the white blood cell by defocussing the red blood cell and substantially focussing the white blood cell. With urine as the fluid sample cellular particles (such as blood cells or epithelial cells) can be separated from casts—the latter being much larger than the former.

A variation of the methods of the present invention would be to flow a second sheath fluid through the flow cell 10. The flow rate of both of the sheath fluids and of the sample fluid are adjusted such that the corners of masses of the particles lie substantially in one plane. Each of the sheath fluids is flowing in a plane substantially parallel to the width of the flow cell 10, with the width many times the thickness.

There are many advantages to the methods of the present invention. First and foremost is that when the particles are in focus, maximum contrast is achieved. The maximum edge intensity of each particle is in the image plane. Furthermore, with the co-planarity of the centers of mass of the particles, the microscope 30 does not have to be adjusted to view each particle. Finally, with the centers of mass of particles lying substantially in one plane, optical separation of the particles can also be achieved. The shift in focal plane can be deliberately affected to diffuse the edge intensity of the smaller particles while the larger particles remain in focus. Furthermore, the shift in the focal plane can cause the diffusion of even all of the particles that are in the one plane 120 and the microscope can be focused on the smaller particles which are not affected by the hydrodynamics of the flow cell 10 which lie in a different plane.

Claims

1. A method of aligning particles in a fluid sample, moving in a directoin through a flow cell, said cell having a width and a thickness, each perpendicular to said direction, each of said particles having a center of mass, said method comprising the steps of:

conveying said fluid sample through said flow cell;

flowing a sheath fluid through said flow cell in said direction; and

adjusting the flow rate of said sheath fluid such that said particles are aligned substantially with their minimum cross-sectional area extended transverse to said direction and their maximum cross-sectional area extended substantially parallel to said width and with the centers of mass of said particles lying substantially in one plane.

2. A method of viewing particles in a fluid sample, moving in a direction through a flow cell, said cell having a width and a thickness, each perpendicular to said direction at an imaging area, a microscope means aligned to view said particles in said imaging area, each of said particles having a center of mass, said method comprising the steps of:

conveying said fluid sample through said flow cell;

flowing a sheath fluid through said flow cell in said direction;

adjusting the flow rate of said sheath fluid such that said particles are aligned substantially with their minimum cross-sectional area extended transverse to said direction and their maximum cross-sectional area extended substantially parallel to said width and with the centers of mass of said particles in said area lying substantially in one plane; and

causing said particles to be in focus by said microscope means.

3. A method of optically separating particles of different sizes in a fluid sample, moving in a direction through a flow cell, said cell having a width and a thickness, each perpendicular to said direction at an imaging area, a microscope means aligned to view said particles in said imaging area, each of said particles having a center of mass, said method comprising the steps of:

conveying said fluid sample through said flow cell;

flowing a sheath fluid through said flow cell in said direction;

adjusting the flow rate of said sheath fluid such that said particles are aligned substantially with their minimum cross-sectional area extended transverse to said direction and their maximum cross-sectional area extended substantially parallel to said width and with the centres of mass of said particles in said area lying substantially in one plane; and

causing said microscope means to be focused on a plane other than said one plane, such that particles of one size are defocused and particles of another size are substantially in focus.

4. A method of aligning particles in a fluid sample, moving in a direction through a flow cell, said cell having a width and a thickness, each perpendicular to said direction, each of said particles having a center of mass, said method comprising the steps of:

conveying said fluid sample through said flow cell;

flowing a sheath fluid through said flow cell in said direction; and

adjusting the flow rate of said fluid sample such that said particles are aligned substantially with their minimum cross-sectional area extended transverse to said direction and their maximum cross-sectional area extended substantially parallel to said width and with the centers of

mass of said particles lying substantially in one plane.

5. A method of viewing particles in a fluid sample, moving in a direction through a flow cell, said cell having a width and a thickness, each perpendicular to said direction at an imaging area, a microscope means aligned to view said particles in said imaging area, each of said particles having a center of mass, said method comprising the steps of:

conveying said fluid sample through said flow cell;

flowing a sheath fluid through said flow cell in said direction;

adjusting the flow rate of said fluid sample such that said particles are aligned substantially with their minimum cross-sectional area extended transverse to said direction and their maximum cross-sectional area extended substantially parallel to said width and with the centers of mass of said particles in said area lying substantially in one plane; and

causing said particles to be in focus by said microscope means.

6. A method of optically separating particles of different sizes in a fluid sample, moving in a direction through a flow cell, said cell having a width and a thickness, each perpendicular to said direction at an imaging area, a microscope means aligned to view said particles in said imaging area, each of said particles having a center of mass, said method comprising the steps of:

conveying said fluid sample through said flow cell;

flowing a sheath fluid through said flow cell in said direction;

adjusting the flow rate of said fluid sample such that said particles are aligned substantially with their minimum cross-sectional area extended transverse to said direction and their maximum cross-sectional area extended susbtantially parallel to said width and with the centers of mass of said particles in said area lying substantially in one plane; and

causing said microscope means to be focused on a plane other than said one plane, such that particles of one size are defocused and particles of another size are substantially in focus.

7. The method of Claims 1, 2, 3, 4, 5 or 6 wherein said one plane is substantially parallel to said width.

8. The method of Claim 7 wherein said width is many times said thickness.

9. The method of Claims 1, 2, 3, 4, 5 or 6 further comprising the step of:

flowing a second sheath fluid through said flow cell.

10. The method of Claims 2, 3, 5 or 6 wherein said causing step is to move the microscope means.

11. The method of Claims 2, 3, 5 or 6 wherein said causing step is to change the flow rate of said sheath fluid.

12. The method of Claims 1, 2, 4 or 5 wherein said fluid sample is blood.

13. The method of Claims 3 or 6 wherein said fluid sample is blood.

14. The method of Claim 13 wherein said one size is red blood cell and another size is white blood cell.

15. The method of Claims 1, 2, 4 or 5 wherein said fluid sample is urine.

16. The method of Claims 3 or 6 wherein said fluid sample is urine.

17. The method of Claim 16 wherein said one size is cellular particle and another size is cast.

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